# Validation data for HEK-Dual™ hTLR9 cells

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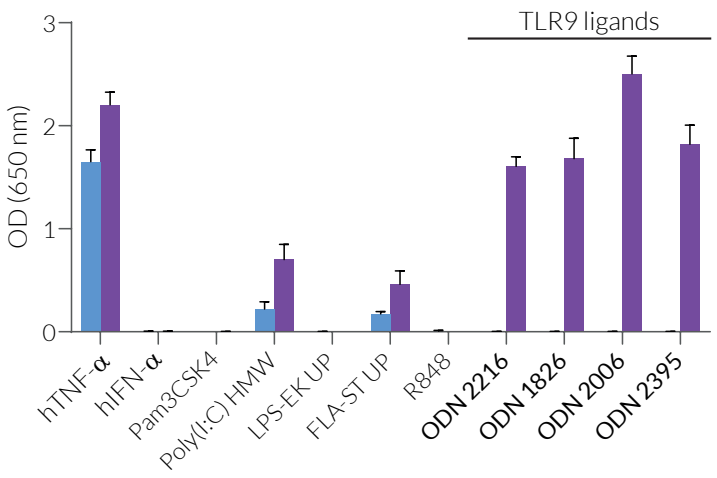
Version 24B09-AK

HEK-Dual™ hTLR9 cells were generated from the HEK-Dual™ cell line through the stable expression of the human Toll-like receptor 9 (hTLR9). These cells feature two reporter genes allowing the simultaneous study of NF-κB- and IRF-induced responses, by monitoring the SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase activities, respectively. Due to the stable expression of hTLR9, these cells show strong NF-κB and IRF responses upon incubation with oligonucleotides containing CpG motifs (ODN CpGs), when compared to their parental cells HEK-Dual™ **(Figures 1 & 2)**. Of note, as HEK293 cells express endogenous levels of TLR3 and TLR5, HEK-Dual™ - derived cells respond to the cognate ligands Poly (I:C) and flagellin, respectively.

## Functional validation of HEK-Dual™-derived cells (NF-κB responses)



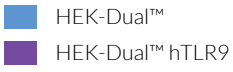
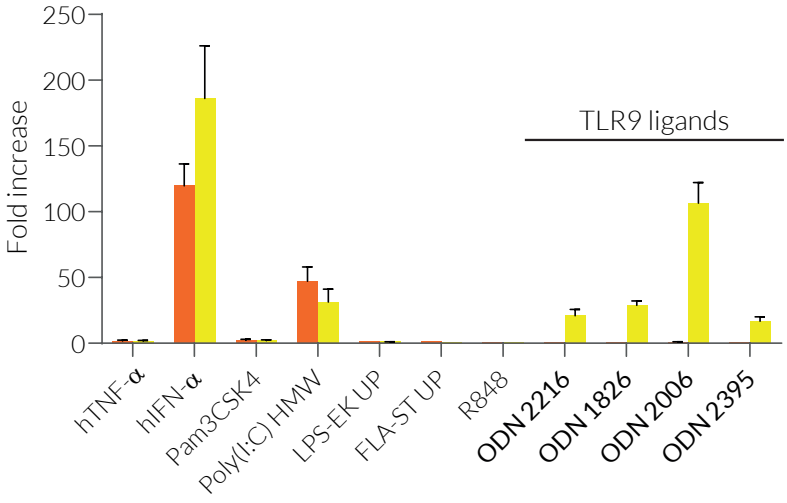




**Figure 1. NF-κB responses in HEK-Dual™ -derived cells.** HEK-Dual™ and HEK-Dual™ hTLR9 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), hIFN-α (IRF-positive control, 1000 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 100 ng/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 1 ng/ml), R848 (TLR7/8 ligand, 10 µg/ml), ODN 2216 (class A, human TLR9-preferred, 10 µM), ODN 1826 (class B, mouse TLR9-preferred, 10 µM), ODN 2006 (class B, human TLR9-preferred, 1 µM) or ODN 2395 (class C, human/mouse TLR9-preferred, 10 µM). After 24h incubation, the NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown as optical density (OD) at 630 nm (mean ± SEM).

## Functional validation of HEK-Dual™-derived cells (IRF responses)



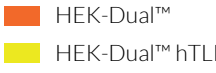




**Figure 2. IRF responses in HEK-Dual™ -derived cells.** HEK-Dual™ and HEK-Dual™ hTLR9 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), hIFN-α (IRF-positive control, 10 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 100 ng/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), R848 (TLR7/8 ligand, 10 µg/ml), ODN 2216 (class A, human TLR9-preferred, 10 µM), ODN 1826 (class B, mouse TLR9-preferred, 10 µM), ODN 2006 (class B, human TLR9-preferred, 1 µM) or ODN 2395 (class C, human/mouse TLR9-preferred, 10 µM). After 24h incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown in fold response over non-induced cells (mean ± SEM).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